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Claude Jolivalt, Catherine Madzak, Agathe Brault, Eliane Caminade, Christian Malosse, et al.. Expression of laccase IIIb from the white-rot fungus Trametes versicolor in the yeast Yarrowia lipolytica for environmental applications. 2nd European Meeting in Oxizymes, Jun 2004, Naples, Italy. 2004. hal-01600125

HAL Id: hal-01600125 https://hal.science/hal-01600125

Submitted on 4 Jun2020

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Expression of laccase IIIb from the white rot fungus *T. versicolor* in the yeast Yarrowia lipolytica for environmental applications



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INTRODUCTION

- Fungal laccases (EC 1.10.3.2) are involved in lignin transformation, morphogenesis, pathogenesis and fungal virulence, as well as in the oxidation of numerous xenobiotics [1-3]
- A wide range of their substrates are of environmental interest, and are recognized pollutants of waters and soils
- Laccases are potential useful tools to improve the chemical quality of polluted media through bioremediation^[1,3]

Our objective is the design of laccases with:

- a high efficiency in catalysis (high redox potential)
- a wider variety of substrates
- a pH of activity optimized for natural media

Our approach is based on the knowledge of the cavity enclosing a weak reducing substrate of the enzyme, the arylamine 2,5-xylidine, and on the replacement of neighboring interacting residues

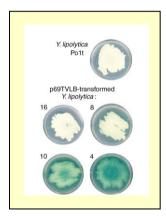
RESULTS: Expression of LacIIIb by Yarrowia lipolytica

2,5-xylidir Structure of LacIIIb [4] (atomic coordinates: pdb accession number 1KYA) • , copper atoms; • and o, glycosylation sites

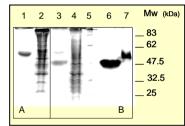
- The gene encoding LacIIIb has been cloned in Escherichia coli using the pGEM®-T plasmid

- It has been sequenced and comprises 1563 bases (sequence accession number AF414109), including a peptide signal

- It was then expressed in the yeast Yarrowia lipolytica using the pINA1269 vector (Patent INRA/INA-PG), thus allowing the production of an active form of the recombinant enzyme (transformation of the substrate ABTS to a green oxidized form on solid culture media and oxidation of guaïcol after electrophoretic separation). After tryptic digestion of the transformed Y. lipolytica extract, the mass spectrum contains the most significative ions detected and assigned to T. versicolor laccase peptides.

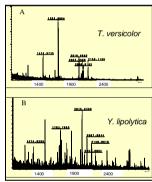


Laccase activity detection on agar plates assay. Numbers refer to different transformant clones of Y. lipolytica Po1g strain having received p69TVLB plasmid.



Electrophoresis analysis of recombinant laccase IIIb. [B] Samples were not boiled before loading on the gel. Lanes 3 and 6 -Laccase IIIb from *T. versicolor*. Lanes 4 and 7 - Concentrated crude cell free Eactase into information of the second secon

was detected in lanes 6 and 7 by coloration with guaïacol



MALDI-TOF spectra

MALDF1 OF Spectra [B] Laccase IIIb from *T. versicolor*. [B] Analysis of the band corresponding to the activity detected in electrophoresis pattern of crude supernat from a p69T/UB-derived transformant (clone 4) of Yarrowia lipolytica expressing laccase IIIb.

APPLICATION: Towards the engineering of fungal laccases

1- T1 site and neighboring 2,5-xylidine ligand

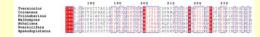


Two charged or polar residue interact with the amino group of the xylidine: His (H458) coordinates the copper that functions

as the primary electron acceptor

Asp (D206) possesses acido-basic properties that could be responsible for the pH-dependent activity towards substrates

2 - Sequence alignment of laccases from fungi and higher plants



Biological roles of laccases from fungi and higher plants appear to be opposite: fungal laccases are involved in ligninolysis whereas enzymes from plants catalyze the initial polymerization of monolignols during lignification

Fungal laccases have a negatively charged residue at the 206 position (Asp, D or Glu, E), whilst those of higher plants have an asparagine (Asn, N)

= Coprinus cinereus: Pcinnabarinus = Pvcnoporus cinnabarinus:

Malbomyces = Melanocarpus albomyces ligher plants: Athaliana = Arabidopsis thaliana; Rvernicifera = Rhus vernicifera; Apeudoplatanus = Acer pseudoplatanus

3- Strategy of directed mutagenesis

- Three mutants have been obtained comprising:
- a residue with another negative chain: D206E
- a residue with a polar uncharged chain: D206N - a residue with an uncharged chain: D206A

-Their catalytic properties are currently studied.

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